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synthetase (tRNA/aaRS) pairs in the presence of target protein mRNA that contains an orthogonal stop codon in the coding sequence and adequate cellular levels of UAA. While conceptually straightforward, many technical barriers impede facile application of this technology in a broad array of eukaryotic expression systems. Cell loading of UAAs is one such obstacle since amino acids usually require specific transporters for cellular uptake. To improve UAA cell loading, the methyl ester of one fluorescent UAA, L-Anap (L-Anap-AM) was tested in eukaryotic expression systems. L-Anap-AM is soluble in ethanol and DMSO, and readily diluted into aqueous solutions. Full length GFP (containing a stop codon mutation at Y39) and AHA2 H^+-ATPase (stop codon mutation at W71) were produced in yeast strains expressing a tRNA/aaRS pair for L-Anap after growth in L-Anap-AM containing media. Both expressed proteins were fluorescent and GFP showed efficient FRET between L-Anap and the protein fluorochrome. LC/MS/MS studies also showed that L-Anap was located at residue 39 in GFP. These studies demonstrated that L-Anap-AM is correctly incorporated into peptide chains during translation. Studies were also carried out in *Xenopus* oocytes in which nuclear injection of the tRNA/aaRS pair for L-Anap was followed by injection of cRNA for Connexin 26 (Cx26) or the Shaker K_V channel containing a stop codon mutation at specific locations. Both Cx26 and K^+ currents were measured in injected oocytes, using a two-microelectrode voltage clamp, only after incubation in an L-Anap-AM containing storage buffer. These studies demonstrate that L-Anap-AM can be used effectively to generate UAA-containing proteins in a variety of eukaryotic expression systems.

3150-Pos Board B580
Dithioamide Peptides and Proteins: Synthesis and Application to Tracking Protein Conformational Changes by Fluorescence Spectroscopy
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Tracking protein conformational change is important to understand the folding and function of proteins. Förster resonant energy transfer (FRET) and photo-induced electron transfer (PET) are widely used to get time-resolved structural information on protein motions. However, the relatively large size of fluorophores and quenchers may introduce significant perturbations to protein structure. The thioamide bond, a single atom substitution of the peptide bond, has recently been shown to be a minimalist fluorescent quencher of various fluorophores by either FRET- or PET- based mechanisms. Unlike commonly used fluorescence probes, thioamides are sufficiently small that they can be placed at nearly any position in the protein sequence without significant alteration of the secondary structure. However, moderate quenching efficiency may limit its sensitivity for some applications. Here, we show that two consecutive thioamide bonds can be incorporated into peptide and protein backbones, and the quenching effect is strengthened compared with a mono-thioamide. Thus dithioamide bonds provide increased sensitivity to detect protein conformational changes and may be used for advanced spectroscopy applications like Fluorescence correlation spectroscopy (FCS) and Fluorescence lifetime imaging microscopy (FLIM).

3151-Pos Board B581
Parallels between Enzyme Action and Tryptophan Fluorescence Brightness in Proteins
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Thousands of scientists studying proteins rely on the intensity, wavelength, and lifetimes of emitted light (fluorescence) from the amino acid tryptophan (Trp) because it is extremely sensitive to its ''environment'' in a protein. But, what exactly does ''environment'' mean? In the last several years, we have combined classical molecular dynamics with simplified quantum mechanics and electrostatics to gain considerable insight into what environments promote and quench Trp fluorescence. Close parallels can be drawn between our simulations of Trp fluorescence brightness and simulations of enzyme effectiveness, especially for the ''single electron transfer'' mechanism. We have carried out MD simulations of Staphylococcal nuclease and ribonuclease T1 and determined the electric potential difference between the phosphorus subject to nucleophilic attack and the putative electrophile. These are characterized by potential differences of 2-3 volts, with fluctuations spanning 1.5 volts, quite similar to the energy gaps between the fluorescing state of Trp and charge transfer states that result in fluorescence quenching. We shall report results for a variety of enzymes representing six major classes of enzymes.

3152-Pos Board B582
Investigation of E. coli Heptosyltransferase I Dynamics
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Interest in new methods to treat gram-negative bacterial infections has emerged due to a significant increase in antibiotic resistance amongst bacteria. Bacterial biofilms are a major contributor to this immunity. Heptosyltransferase-I (HepI) is an essential enzyme for the biosynthesis of lipopolysaccharides (LPS), an important component to bacterial biofilms. Cells deficient in HepI have decreased intestinal colonization and are more susceptible to hydrophobic antibacterials, which makes HepI a good target for developing inhibitors. HepI is a member of the GT-B structural subclass of glycosyltransferases. Crystal structures of GT-B enzymes have been observed to interconvert between open and closed conformations based up the ligation state of the proteins; we therefore hypothesize that HepI will also interconvert between open and closed conformations to enable catalysis. In HepI, there are eight tryptophan residues, which enable us to observe changes in the intrinsic tryptophan fluorescence upon substrate binding. Using wild-type and mutant forms of HepI we are attempting to discern which regions are undergoing conformational changes upon binding of the sugar acceptor substrate (associated with an observed blue shift in the fluorescence). Individual HepI tryptophan residues have been mutated to phenylalanine. Arginine residues that we hypothesize to have an important role in substrate induced conformational changes have also been mutated. Fluorescence circular dichroism have been used to determine the impact of these residues upon binding. Enzyme kinetics were also performed on all mutants to ensure that the mutagenesis was not impacting catalysis. Data thus far suggest that a conformational change is indeed needed for chemistry to occur. Monitoring whether a large dynamic closing occurs, is also being explored using mutagenesis and site specific fluorophore incorporation. Ultimately, an enhanced understanding of HepI's protein dynamics and mechanism may lead to the design of more effective gram-negative therapeutics.

3153-Pos Board B583
The Role of Chaperone Proteins in Cataract Aggregation: A Two-Dimensional Infrared Study
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Crystallin proteins need to maintain their native structures at high concentration in the lens to enable vision. When these proteins aggregate into opaque deposits, cataracts are formed. Most kinds of isolated crystallin proteins form amyloid fibril structures when treated with denaturants in vitro, but these structures have not been confirmed from examination of a cataractous lens. It is hypothesized that alpha crystallins form molecular chaperones that bind to unfolded proteins and interrupt the aggregation pathways. We study the aggregation of gammaD-crystallin in the presence of alphaB-crystallin with two-dimensional infrared (2D IR) spectroscopy that is sensitive to the secondary structure of proteins. Using 13C isotope labeling of either the gammaD-crystallin or the alphaB-crystallin, we can independently watch structural changes in both proteins simultaneously. In addition, cross-peaks in the 2D IR spectra reveal coupling between the different proteins. Using these tools, we have observed interaction between alphaB-Crystallin and aggregated gammD-Crystallin. Our study provides a new way of monitoring protein-protein interactions and will be valuable in the further studies of molecular chaperone interactions with disease-related protein aggregates and their intermediates.

3154-Pos Board B584
Development of a Vibrational Hydration Ruler
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Amino acids covalently modified with spectroscopic reporters offer the potential to probe local protein hydration with high spatial and temporal resolution when coupled with the appropriate spectroscopic technique. Three azidophenylalanine residues have been synthesized and, in combination with the commercially available 4-azido-L-phenylalanine, form a series of unnatural amino acids (UAAs) containing the azide vibrational reporter at varying distances from the aromatic ring of phenylalanine. The azide vibrational reporter was selected due to the position, sensitivity and extinction coefficient of the azide asymmetric stretch vibration. The sensitivity of the azide reporters for these UAAs was investigated in solvents that mimic distinct local protein environments. Three of the four azido modified phenylalanine residues were successfully genetically incorporated into a surface site in superfolder green fluorescent protein (sfGFP) utilizing an engineered, orthogonal